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Identification of weeping crabapple cultivars by microsatellite DNA markers and morphological traits

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Abstract

Ornamental crabapples are small landscape trees with charming flowers, colourful fruits and many growth forms. The first weeping crabapple cultivars, *Malus prunifolia* 'Pendula' and 'Pendula Nova', were described in Sweden around 150 years ago. Our study was aimed at identification and characterization of weeping crabapple clones by microsatellite markers and morphological traits. We analysed 13 Swedish and Finnish trees and 8 reference accessions including *Malus prunifolia* 'Pendula' and three international cultivars belonging to its progeny. The 21 trees represented 13 distinct genotypes. Five local trees were identified as the historical 'Pendula', assumed to be extinct from the nursery trade. On grounds of morphological traits and historical records, two old Swedish trees were concluded to represent 'Pendula Nova'. The authenticity of the trees could not be confirmed by DNA markers because no known plant of the old cultivar was found in botanical collections. The Finnish clone 'Hyvingiensis' proved unique among the crabapple accessions studied. 'Hyvingiensis' was probably raised from seed at the Finnish State Railways Nurseries about 110 years ago. Several mislabellings were revealed among both the local and the reference samples. A novel identification key was created to aid discrimination between the clones by their morphological traits. A combination of DNA fingerprints, comparison of morphological traits and tracing information in relevant archives and old garden literature proved useful for solving the origin and identity of weeping crabapples. The results contribute to conservation of garden plants and stabilization of horticultural nomenclature.

Keywords:

Cultivar identification, fingerprinting, landscape plant, *Malus*, morphological trait, SSR.

1. Introduction

Ornamental crabapples (*Malus* spp.) are small trees and shrubs valued for their charming flowers, handsome summer and autumn foliage, and colourful fruits that often remain on the tree long after leaf fall. Crabapples provide a wide range of growth forms as well as flower and fruiting characteristics. Many crabapples are more winter hardy than the common apple (*Malus domestica*) and the fruits of several cultivars are suitable for economic uses; facts that have certainly raised the interest in growing crabapples at high latitudes.

Crabapples are distinguished from apple trees grown for fruit production by fruit size: members of the genus *Malus* that have fruits 2 inches (ca. 5 cm) or less in diameter, are considered as crabapples (Wyman, 1955). Some wild *Malus* species and varieties are grown as ornamental crabapples, but most of the cultivated forms are hybrids between different *Malus* taxa. In the 20th century, breeding of ornamental crabapples was carried out mainly in Canada and in the U.S.A, where approximately 400 to 600 different forms and cultivars are grown (Dirr, 2009).

A special group of ornamental crabapples is those with pendulous branches, the weeping or hanging cultivars. The first two weeping *Malus* varieties described in horticultural literature arose in 1860 and 1873 at the experimental station of the Royal Swedish Academy of Agriculture in Stockholm (Lindgren, 1878a, 1878b). The clones were named as *Pyrus prunifolia pendula* and *P. prunifolia pendula nova*. The earlier of them, *Malus prunifolia* 'Pendula' according to current nomenclature, proved to be very winter hardy (up to 66° lat. N) and was soon introduced to tree nurseries in Europe and North America (Lindgren, 1878a, 1878b).

The classic weeping crabapple cultivars, 'Excellenz Thiel' (introduced by the German nursery L. Späth in 1909), 'Oekonomierat Echtermeyer' (introduced by Späth in 1914) and 'Red Jade' (introduced by the Brooklyn Botanic Garden in 1953), belong to the first- and

second-generation progeny of *Malus prunifolia* ‘Pendula’. ‘Excellenz Thiel’ is a seedling of *M. p.* ‘Pendula’, with *M. floribunda* as the putative pollen parent (Späth, 1909), while ‘Red Jade’ is an open pollinated seedling of ‘Excellenz Thiel’ (Jefferson, 1970). The pink-flowering ‘Oekonomierat Echtermeyer’ is described as a hybrid between ‘Excellenz Thiel’ and *Malus pumila* var. *niedzwetzkyana* (Jefferson, 1970). *Malus* ‘Elise Rathke’ (first recorded in 1884 and in our opinion correctly named as *Malus domestica* ‘Pendula’) is another old weeper that is not a crab, but rather a common apple on grounds of fruit diameter, 5.9 cm in average, according to the database of UK National Fruit Collections (http://www.nationalfruitcollection.org.uk).

In the second half of the 20th century, some 50 new weeping crabapple cultivars were released (Fiala, 1994). Nearly all the new weepers were bred in the U.S.A. and only a few of them are marketed in Europe, to judge from the Plant Finder of the Royal Horticultural Society (http://www.rhs.org.uk/plants/) and the name list of the European Nurserystock Association (http://www.internationalplantnames.com/).

The two oldest weepers, *M. prunifolia* ‘Pendula’ and ‘Pendula Nova’, have not been available in Sweden for a long time. Andréasson and Wedelsbäck Bladh (2011) noted both cultivars in the catalogues of two Swedish nurseries between 1864 and 1900, but not thereafter. From an historical inventory list (Anon. 1879), we know that both were imported to Finland before the end of the 19th century. *M. prunifolia* ‘Pendula’ was cited in Finnish garden literature until the 1940s (e.g. Sorma, 1932; Schalin, 1935) and marketed by local nurseries until the early 1960s (e.g. Harviala plantskolor och växthus, 1939; Olsson, 1962). Most Finnish and a few Swedish tree nurseries currently offer a white-flowering crabapple with strongly weeping branches, named as *Malus* ‘Hyvingiensis’. The cultivar was first mentioned in garden literature in 1953 (Lehtonen and Jokela, 1953) and it was originally

spread by the Finnish State Railways Nurseries (active 1873-1990 at Hyvinkää), but the origin and uniqueness of 'Hyvingiensis' remain uncertain.

The aim of our research was to identify and characterize weeping crabapple clones cultivated in old parks in Sweden and Finland. Our specific objectives were (1) to find out if extant plants of the Swedish cultivars, 'Pendula' and 'Pendula Nova', can still be found, (2) to determine the cultivar identity of the Finnish clone 'Hyvingiensis' and (3) to confirm cultivar names of a few weeping crabapples grown in Swedish and Finnish botanical collections. For cultivar identification we used microsatellite (SSR) based DNA markers and morphological traits.

2. Materials and methods

2.1 Plant material

The 13 local accessions studied (Table 1) involved un-named crabapple trees recorded in connection with park inventories or through observations of plant experts as well as a few doubtfully named trees in botanical gardens or arboreta. For 'Hyvingiensis', an about 65 years old tree of documented origin at the Finnish State Railways Nurseries, was used as the authentic accession and a tree propagated by Ahonen's Nursery represented plant material currently sold as 'Hyvingiensis'. Reference samples of the weeping cultivars 'Elise Rathke', 'Excellenz Thiel', 'Oekonomierat Echtermeyer', *M. prunifolia* 'Pendula' and 'Red Jade' were obtained from botanical gardens in Europe and North America (Table 1). *M. prunifolia* 'Pendula Nova' could not be traced in any of the 25 plant collections that were asked. Instead, we received a sixth reference sample of *M. baccata* 'Pendula' from the Botanical Garden of the Tartu University.

98 Young leaf samples of the Swedish and Finnish trees were collected in 2009 and 2012.
99 Reference samples were acquired in 2009, 2010 and 2012. All samples were shipped to the
100 Department of Agricultural Sciences, University of Helsinki, sealed in plastic bags and stored
101 at -20°C until DNA analysis, performed in 2012.

Table 1
The list of weeping crabapple accessions genotyped.

Accession name	Location	Age and origin
1 *Malus* sp.	Sunnersta manor, Uppsala, Sweden	Ca. 100-year-old tree in the park of the manor house, origin unknown.
2 *Malus* sp.	Ulleråker hospital, Uppsala, Sweden	Ca. 130-year-old tree in the hospital park, origin unknown.
3 *Malus* sp.	Mattias Iwarsson, Uppsala, Sweden	A young graft on A2 from a ca. 50-year-old tree of unknown origin in the Botanical Garden, Uppsala University, Sweden.
4 *Malus* sp.	Mattias Iwarsson, Uppsala, Sweden	A young graft on A2 from a ca. 130-year-old tree of unknown origin at the Park Maria Kronbergs Minne in Falun, Sweden.
5 *Malus* sp.	Lindesberg church square, Sweden	Ca. 100-year-old tree at the square close to the church, origin unknown.
6 *Malus* 'Hyvingiensis'	Hyvinkää Railway Park, Finland	Planted in 1953, originating from the Finnish State Railways Nurseries.
7 *Malus* "Hyvingiensis"[a]	Ahonen's Nursery, Karttula, Finland	A young tree grafted on seedling rootstock, scion originating from a mother plant of Ahonen's own.
8 *Malus* sp.	Kauppilanaukio Park, Hyvinkää, Finland	Planted in 1997, purchased from an unknown Finnish nursery.
9 *Malus* sp.	Esplanadi Park, Helsinki, Finland	Planted in 1998, purchased from Sundberg Nurseries, Lohja, Finland.
10 *Malus* sp.	Annanpuisto Park, Tuusula, Finland	Planted in 1998, purchased from an unknown Finnish nursery.
11 *Malus* hybr. 1952-3893	Gothenburg Botanical Garden, Sweden	Received in 1952 from Magnus Johnson's Nursery, Södertälje, Sweden, a putative hybrid between *M.* ×

		zumi ‘Calocarpa’ and *M.* ‘Oekonomierat Echtermeyer’
12 *Malus* “Elise Rathke” [a] 1990-0214	Botanic Garden of the University of Turku, Finland	Planted in 1990, purchased from Viksten’s Nursery, Tammela, Finland.
13 *Malus* “Red Jade” [a]	Kellokoski-Ohkola Arboretum, Tuusula, Finland	Planted in 1998, purchased from Lalla’s Nursery, Järvenpää, Finland.

Reference cultivars with accession numbers:

14 *Malus prunifolia* 'Pendula' 1982.0273*A	Sir Harold Hillier Gardens, UK	Age and origin unknown.
15 *Malus prunifolia* 'Pendula'[b]	Dubrava Arboretum, Lithuania	Planted in 2001 or 2002, origin unknown.
16 *Malus* 'Excellenz Thiel' H901501	RHS Garden, Hyde Hall, UK	Planted between 1955 and 1993, origin unknown.
17 *Malus* ‘Oekonomierat Echtermeyer’ 9069-1937A	Montreal Botanical Garden, Canada	Received from Herm. A. Hesse Nursery, Weener-Ems, Germany.
18 *Malus* 'Red Jade' 532-75	Arnold Arboretum, USA	Received from Ruth Birkhoff, Cambridge, USA.
19 *Malus* 'Elise Rathke' 56-99	Arnold Arboretum, USA	Received from Jac. Jurissen & Sons Nursery, Naarden, the Netherlands.
20 *Malus* 'Elise Rathke' 1977.3941*Z	Sir Harold Hillier Gardens, United Kingdom	Age and origin unknown.
21 *Malus baccata* 'Pendula'[b]	Botanical Garden of Tartu University, Estonia	Age and origin unknown.

[a] Cultivar names to be confirmed are enclosed in double quotation marks to set them apart from the accessions of the reference panel.
[b] Accession number not available.

98 *2.2. DNA isolation and SSR analysis*

99

100 For DNA extraction, 1 cm^2 of leaf tissue per genotype was ground to a fine powder with
101 a Retsch Mixer Mill MM400 (Retsch GmbH, Germany). Genomic DNA was extracted using
102 E.Z.N.A.® plant DNA kit (Omega Bio-Tek Inc., USA) following the supplier's instructions.
103 The DNA isolates were kept at -20°C before polymerase chain reactions (PCR).
104 Seven SSR primer pairs, originally developed for the domestic apple (Gianfranceschi et
105 al., 1998; Liebhard et al,. 2002), were used: CH01d03, CH01h02, CH02c06, CH02c09,
106 CH02c11, CH02d08 and COL. All chosen primers have been utilized for varietal
107 identification of apple genotypes (e.g. Laurens et al., 2004; Guarino et al., 2006; Garkava-
108 Gustavsson et al., 2013). The forward primers were fluorescently labelled with HEX®, FAM®
109 or TET® dyes.
110 For PCR amplifications 2 µl of genomic DNA was used in an 8 µl reaction containing
111 10× reaction buffer (KCl), 1.5 mM $MgCl_2$, 2.0 mM dNTP mixture, 0.3/0.6 U Taq DNA
112 polymerase (Finnzymes, Thermo Fisher Scientific, USA) and 0.3 µM of both primers. The
113 PCR reactions were performed using a Mastercycler gradient (Eppendorf) with the following
114 thermal program: (1) 95 °C for 2 min, (2) 28 cycles of 94 °C for 20 s, 55 or 60 °C (depending
115 on the primer) for 40 s and 65 °C for 45 s, (3) 65 °C for 5 min.
116 The PCR products were separated in the Sequencing Laboratory of the Institute of
117 Biotechnology, University of Helsinki, by a capillary electrophoresis system (3730 DNA
118 Analyzer; Applied Biosystems Inc., USA) and fragment sizes were determined by Peak
119 Scanner Software 1.0 (Applied Biosystems Inc., USA). In cases where only one peak was
120 visible, its size was recorded twice since the locus was considered homozygous.

121

122 *2.3. Morphological traits*

123

124 The local accessions were examined for their morphological characteristics, adopted
125 from the descriptor list for ornamental crabapples published by the International Union for
126 the Protection of New Varieties of Plants (UPOV, 2003). Measurements were taken on 3-10
127 well developed leaves, flowers and fruits. To separate between the accessions in a key,
128 observations on a set of additional characters (not UPOV) were used. No morphological traits
129 were recorded for tree no. 7.

130 The plants were documented by taking digital images of both the general appearance and
131 morphological details. Dried voucher specimens of the Swedish trees are preserved at the
132 Museum of Evolution, Uppsala University and those of the Finnish trees are kept at the
133 Herbarium of the Finnish Museum of Natural History, University of Helsinki.

134

135 *2.4. Data analyses*

136

137 The number of alleles per locus, the effective number of alleles and the observed and
138 expected heterozygosity were computed from the allelic profiles. For calculation of genetic
139 distances between the accessions, the allele sizes were transformed into binary scores because
140 two of the accessions appeared to be triploid. The measures of genetic variation and the
141 genetic distances were calculated with GenAlEx 6.5 (Peakall and Smouse, 2012). For
142 visualization of the similarity relationships, a dendrogram was constructed by the unweighted
143 pair-group method with arithmetic average (UPGMA) in the MEGA software version 5.10
144 (Tamura et al., 2011)

145 The cultivar identity of the 13 local accessions was investigated by comparing their DNA
146 profiles with those of the 8 reference samples. The assumed parent-offspring relationships
147 were checked by comparing the multilocus allelic profiles of the putative relatives. The

morphological traits were used to confirm the cultivar identifications indicated by allelic data. The phenotypic differences between the local accessions were summarized in an identification key.

3. Results and discussion

3.1. SSR polymorphism

The primer pairs CH01h02 and CH02c11 each amplified two different loci, one of which was monomorphic in all accessions. The monomorphic loci were discarded from data analyses. The seven polymorphic loci amplified 62 alleles in the entire set of accessions studied. The two triploid accessions displayed four unique alleles not found in the diploid genotypes. In the 19 diploid genotypes, the average number of alleles per locus was 8.3 (Table 2), which is somewhat less than the average allele number (9.3) in the 85 traditional Swedish and Finnish domestic apple cultivars studied by Garkava-Gustavsson et al. (2013). Other studies on domestic apple germplasm collections revealed an average of 9.2 alleles (Guarino et al., 2006) and 9.7 alleles (Gasi et al., 2010) per locus, respectively. The numbers of both accessions and SSR loci were smallest in our study, which may explain the differences.

Table 2
Observed variation at 7 microsatellite loci tested in 19 diploid weeping crabapple accessions. Accessions showing an identical or triploid allelic profile were excluded from calculation of the diversity parameters.

SSR locus	Size range (in base pairs)	Number of alleles	Effective number of alleles	Observed heterozygosity	Expected heterozygosity

CH01d03	127-189	10	5.65	1.00	0.82
CH01h02	227-273	10	6.86	1.00	0.85
CH02c06	216-252	7	6.00	0.92	0.83
CH02c09	231-259	7	4.97	0.75	0.80
CH02c11	201-243	9	5.24	1.00	0.81
CH02d08	204-257	9	5.88	0.92	0.83
COL	215-241	6	3.51	0.50	0.72
Mean		8.3	5.44	0.87	0.81

168

169 In the present set of accessions, the expected heterozygosity varied from 0.72 to 0.85 per
170 locus (Table 2) reflecting the high frequency of cross-pollination and self-incompatibility in
171 the genus *Malus*. The mean expected heterozygosity values reported for traditional domestic
172 apple cultivars range from 0.74 (Garkava-Gustafsson et al., 2013) to 0.81 (Guarino et al.,
173 2006).

174

175 *3.2. Morphological traits*

176

177 All 13 local accessions had single, white flowers often with a shade of pink on unopened
178 flower buds and on the outer side of petals. 'Hyvingiensis' (no. 6) and "Red Jade" (no. 13)
179 had large flowers with an average diameter more than 6 cm, while the flowers of the other
180 accessions were of medium size, approx. 5 cm in diameter.
181 The leaves of the local accessions were green and unlobed. The leaf margin was crenate to
182 serrate in all accessions except for 'Hyvingiensis' that had doubly serrate leaf margins.
183 Accessions no. 11 and 13 had small fruits with a deciduous calyx. The other trees had
184 medium-sized fruits with a persistent calyx, apart from tree no. 8, some fruits of which shed
185 their calyx at an early stage. For all local accessions, the predominant colour of the mature
186 fruit skin was yellow with a varying amount of orange or red blush on the exposed side.

187 The morphological characteristics distinguishing the local accessions were summarized as
188 an identification key (Fig. 1). Four accessions were relatively easy to discriminate: in
189 accession no. 2 the fruit stalk was remarkably short, no. 6 was distinguished by its large, pure
190 white flowers and thick leaves with doubly serrate margins, and nos. 11 and 13 had small
191 berry-like fruit. The remaining accessions were distinguishable only by the minor differences
192 in their vegetative parts (Fig. 1).

193

Fig. 1. Identification key to the weeping ornamental crabapples studied for their morphological traits.

1. Fruit with deciduous calyx……*Malus* hybrid, no. 11 and *Malus* "Red Jade", no. 13
Fruit with persistent calyx……..…….…….**2**
2. Fruit stalk short, 2–16 mm……..……....*Malus* sp., no. 2
Fruit stalk long 16–50 mm, often with two glands present at the base….……...…….**3**
3. Leaf margin doubly serrate, flower buds white……..*Malus* 'Hyvingiensis', no. 6
Leaf margin crenate to serrate, flower buds pinkish…..……...……....**4**
4. Young shoots and buds grey or brown....*Malus prunifolia* 'Pendula', nos. 5, 8, 9, 10 and 12
Young shoots and buds brownish-red to red……..……..…….**5**
5. Blade length of the 4th to 6th leaf < 6 cm…..……..*Malus* sp., no. 1
Blade length of the 4th to 6th leaf > 6 cm……....……..….*Malus* sp., no. 3

194

195 *3.3. Cultivar identification*

196

197 The 13 local accessions represented six distinct genotypes. One Swedish (no. 5) and four
198 Finnish trees (nos. 8, 9, 10 and 12) displayed an SSR profile identical with the reference
199 cultivar no. 14, *M. prunifolia* 'Pendula' from the Sir Harold Hillier Gardens. The
200 morphological traits of the five local accessions were similar, with fruit size and colour
201 matching those of *M. prunifolia* 'Pendula' as described by Lindgren (1878a, 1878b).
202 However, our reference panel included another accession (no. 15) named as *M. prunifolia*
203 'Pendula', but its allele profile differed from that of the previous accessions (Table 3).
204 Because fruits of accession no. 15 from the Dubrava Arboretum proved to be dissimilar

205 (small and always without calyx) from Lindgren's description of *M. prunifolia* 'Pendula', the
206 Dubrava tree was regarded as mislabelled.
207 Unfortunately, the origin of *M. prunifolia* 'Pendula' from the Sir Harold Hillier Gardens is
208 unknown. Nevertheless, the authenticity of the six 'Pendula' trees is supported by an
209 additional Swedish *Malus prunifolia* 'Pendula' accession that has grown at the Bergius
210 Botanic Garden, Stockholm since 1925 at the latest, and was discovered late in our study. The
211 morphological characteristics of this accession were consistent with those of the five previous
212 'Pendula' trees. The Bergius Botanic Garden is located next to the grounds once occupied by
213 the experimental station of the Royal Swedish Academy of Agriculture (Lange, 2000), where
214 *Malus prunifolia* 'Pendula' originated.
215 While looking for *Malus prunifolia* 'Pendula' in local plantings, we quite unexpectedly
216 found four relatively young Finnish trees representing this 150-year-old cultivar, which has
217 not been listed in domestic nursery catalogues for 50 years. Practically all apple trees planted
218 in Finland are produced by local nurseries, as the cold climate restricts cultivation of most
219 international cultivars. It seems probable that some Finnish nurseries hold mother stock of
220 *Malus prunifolia* 'Pendula', mislabelled and sold most likely as *Malus* 'Hyvingiensis', the
221 only white-flowering weeper common in present-day Finnish nursery trade.

Table 3

Allele sizes (in base pairs) at seven loci for 14 weeping crabapple accessions analysed in this study. Allelic profiles are not shown for the remaining 7 accessions that were identical to those of other local or reference trees.

	SSR primer pairs						
Accession name	CH01d03	CH01h02	CH02c06	CH02c09	CH02c11	CH02d08	COL
Local accessions							
1 *Malus* sp.	141:150	252:254	250:252	244:244	217:229	212:250	215:231
3 *Malus* sp.	142:150	248:250	250:252	244:244	217:229	212:250	231:231
4 *Malus* sp.	142:158	237:250	242:252	233:250	205:229	212:218	231:231
6 *Malus* 'Hyvingiensis'	137:141:150	235:237:239	242:246	233:250	225:229:233	212:218	229:231:233
11 *Malus* hybr.	129:150	250:273	216:250	231:246	201:215	212:226	215:241
13 *Malus* “Red Jade”	139:150	227:239	238:246	231:250	215:229	214:214	231:231
Reference accessions							
14 *Malus prunifolia* 'Pendula'	141:150	239:254	238:246	233:250	215:233	204:214	231:241
15 *Malus prunifolia* 'Pendula'	127:150	250:252	216:238	231:233	215:229	214:216	241:241
16 *Malus* × *scheideckeri* 'Excellenz Thiel'	137:141	237:254	216:250	231:254	205:229	216:226	231:231
17 *M.* × *gloriosa* 'Oekonomierat Echtermeyer'	150:189	237:254	216:216	231:246	215:243	212:214	241:241
18 *Malus* 'Red Jade'	127:160	231:254	238:248	231:231	223:229	214:216	237:241
19 *Malus* 'Elise Rathke'	137:141	237:242	248:252	246:259	207:217	227:257	221:237
20 *Malus* 'Elise Rathke'	135:139	237:242	–	246:259	207:217	227:257	221:237
21 *Malus baccata* 'Pendula'	127:150	227:254	238:246	231:246	207:229	204:214	215:235

227 The two ‘Hyvingiensis’ accessions (nos. 6 and 7) had identical DNA profiles, unique from
228 the rest of the accessions analysed (Table 3). Three distinguishable alleles were determined at
229 four loci in both ‘Hyvingiensis’ samples, indicating triploidy of the clone. The field
230 observations also were consistent with polyploidy: ‘Hyvingiensis’ has thick, large leaves,
231 large flowers and big fruits with poor seed set.
232 The results of this study proved the authenticity of ‘Hyvingiensis’ plants currently grown
233 by one local nursery. In an earlier SSR analysis, four ‘Hyvingiensis’ plants from four
234 different nurseries were shown to be identical with the 65-year-old tree no. 6 of known origin
235 (Vuorinen, 2012). The name *Pyrus prunifolia pendula* ‘Hyvingiensis’ seems to have been
236 recorded for the first time in an old inventory list, preserved in the archive of the Finnish
237 State Railways Nurseries, that was written between 1893 and 1903 by the then head gardener.
238 The cultivar name is quoted in the same form in a contemporary article on the Finnish State
239 Railways Nurseries (Kornman, 1904). Another report of the same year refers to “a weeping
240 crabapple grown from local seed at the State Railways Nurseries” (Karsten, 1904).
241 ‘Hyvingiensis’ seems thus to have arisen around the turn of the previous century, though it
242 was not described until the early 1950s (Lehtonen and Jokela, 1953).
243 Two of the un-named Swedish accessions (nos. 2 and 4) gave the same band profile for all
244 but one marker (CH01h02), where no fragment was amplified from accession no. 2. The two
245 accessions were assumed to be identical. Another two of the Swedish accessions (nos. 1 and
246 3) had similar allele sizes at four loci, but slightly different bands at the remaining three loci
247 (Table 3). The accessions could not be properly compared by their morphological traits
248 because tree no. 3 was a young graft with few flowers and fruits. Judging from the vegetative
249 characteristics and the origin of the trees, accessions no. 1 and 3 seem to represent different
250 genotypes.

One of our original targets was to determine if *Malus prunifolia* 'Pendula Nova' can still be found in old plantings. The fruit size, colour and taste of accession no. 2 seemed to match Lindgren's short description of 'Pendula Nova' (Lindgren, 1878a, 1878b). The original growing sites of accessions no. 2 and no. 4 are both parks constructed in the 1880s. The Ulleråker hospital tree no. 2 was described 60 years ago in a dendrological publication as "an old, unidentified weeping crabapple" (Hylander, 1955). In its early days the hospital ran a plant nursery that sold seed and living plants to the public. In 1888, the nursery's catalogue offered both old Swedish weepers ("*Pyrus prunifolia pendula* and *P. prunifolia pendula nova")* for sale (Anon., 1888). From these morphological and historical data, we suggest that accessions no. 2 and 4 may very likely represent the historical 'Pendula Nova'.

The hybrid accession no. 11 from Gothenburg Botanical Garden shared at least one allele with its presumed parent cultivar 'Oekonomierat Echtermeyer' at all but one locus (Table 3). The small difference (4 base pairs) might have originated from a mutation in either the parental or our reference accession of *Malus* 'Oekonomierat Echtermeyer', or have arisen from an experimental error. Further parent-offspring relationships were sought for reference sample no. 16, supposed to represent the cultivar 'Excellenz Thiel' that is reported to be both a seedling of *M. prunifolia* 'Pendula' and a parent of both 'Red Jade' and 'Oekonomierat Echtermeyer'. Nevertheless, the microsatellite allele profile of the reference accession no. 16 did not lend support to any of these relationships (Table 3). The small discrepancies between the allele profiles of the two 'Elise Rathke' accessions (Table 3) were probably due to an error in fragment sizing (for marker CH01d03) and a failure in PCR amplification (for marker CH02c06).

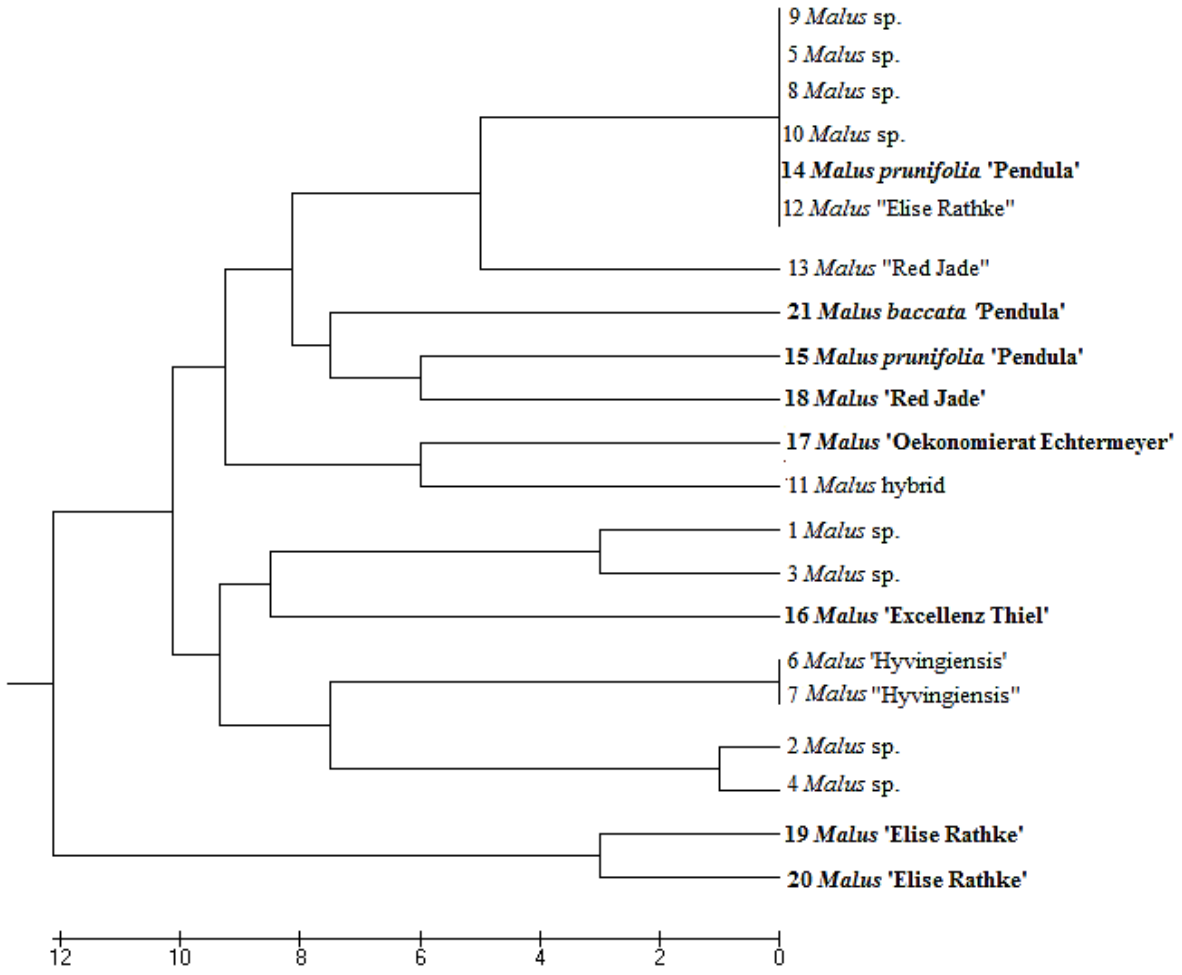
In our data, three local trees (nos. 1, 3 and 13) did not match any of the reference cultivars, and two reference accessions (nos. 15 and 16) seemed not to be true to their names. These five samples represented five unique genotypes. The 100-year-old tree no. 1 could be a local

seedling, and the same may hold true for tree no. 3 that was originally discovered as a graft in an apple hedge. The remaining three trees may belong to cultivars that were not included in the reference panel, or may be seedlings. As pointed out by Jefferson (1970) and Fiala (1994), misnamed crabapples are common in the nursery trade, and one reason for mistaken naming lies in the propagation of cultivars by seed.

In the dendrogram (Fig. 2), the two accessions representing the domestic apple cultivar 'Elise Rathke', form a group of their own, and the proper ornamental crabapples are arranged in two clusters. *Malus prunifolia* 'Pendula', its synonyms and descendants comprise one group that also includes M. *baccata* 'Pendula' and the two small-fruited, mislabelled accessions no. 13 and 15 (Fig. 2). The second crabapple group consists of the four old Swedish accessions, the two 'Hyvingiensis' trees and the presumably misnamed 'Excellenz Thiel' from the RHS Garden Hyde Hall (Fig. 2).

The genetic relationships displayed by the dendrogram (Fig. 2) match the known parentage relations of the cultivars. The morphological traits of fruits are also in agreement with the clustering in Fig. 2: the large-fruited domestic apple accessions form a distinct group, while the first crabapple group is composed of accessions with relatively small fruit and either deciduous or persistent calyx and the second group includes those accessions with larger fruit and persistent calyx. However, as the dendrogram is based on only seven SSR loci per genotype, it could be unstable, and a small change in fingerprint data could alter the topology of the tree.

Fig. 2. UPGMA dendrogram of 21 weeping crabapple accessions based on a genetic distance matrix calculated for codominant data, using seven SSR loci. The reference accessions from botanical collections are printed in bold and cultivar names to be confirmed are enclosed in double quotation marks.



5. Conclusions

In this study we were able to identify several old weeping crabapple clones by a combination of microsatellite genotyping, morphological observations, old garden literature and archival research. Living plants of two valuable historical weepers, one of which is the ancestor of three classic crabapple cultivars, were discovered, the origin and authenticity of one domestic cultivar was solved, and the three clones will be preserved in the national gene banks. As indicated by our results, parks and plant collections may hold historically valuable, though unidentified or misnamed garden plant genotypes. The methods presented here could

be applied to other ornamental plant groups for stabilization of horticultural nomenclature and for conservation of garden plant genetic resources

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